

Parasitological Contamination in Organic Composts Produced with Sewage Sludge

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1. Introduction

The activities performed daily by humans generate large volumes of waste from various areas, increasing environmental pollution and public health problems. According to the recommendations of the World Health Organization (WHO), the reactor effluent treatment from domestic sewages can be used in agriculture, since it applied to cultures that provide little risk of contamination with pathogens (Ayres & Mara, 1996). The sewage sludge is classified as class A when origins of processes with effective reduction of pathogens and can be used without restrictions in horticulture, and in the class B if results of processes of moderate reduction of pathogens, with more restricted use and be applied in reforestation and other cultures in which the risk of environmental and human contamination can be better controlled (Fernandes, 2000; David, 2002).

Sewage sludge must have characteristics that allow its setting within the parameters set for each class (David, 2002). For class A, the most probable number of coliforms per gram of dried sludge must be less than 1000, and the parasitic contamination should be less than one viable egg of helminths in four grams of dried sludge and less than one egg per litre of effluent (WHO, 1989; Fernandes, 2000).

The processes most often employed for the stabilization of sewage include the aerobic and anaerobic digestion. The application of lime and the composting are also recommended in some countries like USA, France and Brazil. However, the efficiency of the stabilization processes depends of the operational quality and of the pathogen characteristics present in the sewage sludge (Paulino et al., 2001).

2. Parasites presented in sewage sludge

Several countries have researched alternatives for final disposal of the waste from water sewage and sludge treatment. The sewage, prior to the stabilization treatment and disinfection, can contain macro and micronutrients and many pathogenic microorganisms and parasites. The handling and use of sewage and sludge obtained, without prior treatment, may promote severe infection to humans and animals (Paulino et al., 2001). According to WHO, 25% of the world's hospital beds are occupied by patients with diseases

transmitted by contaminated water. About 1.5 billion people are infected with *Ascaris lumbricoides* and 1.3 billion with *Ancylostoma* spp. infection (Crompton, 1999).

The establishment of maximum concentrations of viable eggs of helminths in sewage sludge has been the criterion worldwide used to allow the agricultural use of this material (Capizzi-Banas & Schwartzbrod, 2001). Epidemiological studies have shown that the high frequency of helminths in human population, the long survival of the eggs in the environment and the low infectious dose are risks associated with agricultural use of sewage sludge (Soccol et al., 1997). In the world 3.5 billion people are infected with helminths or protozoa and children are the most frequently contaminated, resulting in approximately 60 000 deaths associated with *Ascaris lumbricoides* each year and 70 000 with *Entamoeba histolytica* (WHO, 2000). Table 1 showed the main pathogenic helminths eliminated in human faeces.

Helminths	diseases	cycle characteristics
<i>Ancylostoma duodenale</i>	ancylostomiasis	human – soil – human
<i>Ascaris lumbricoides</i>	ascariasis	human – soil – human
<i>Necator americanus</i>	necatorosis	human – soil – human
<i>Trichuris trichiura</i>	trichuriasis	human – soil – human
<i>Taenia saginata</i>	taeniasis	human – cattle – human
<i>Taenia solium</i>	taeniasis	human – pigs – human
<i>Taenia solium</i>	cysticercosis	human – soil – human

Roque, (1997).

Table 1. Main pathogenic helminths from human faeces

3. Sewage sludge stabilization

The sludge stabilization is used primarily to reduce substantially the numbers of pathogenic organisms, to minimize health risks, controlling odours and to restrict the possibility of further decomposition. The stability is generally associated with the tendency to biodegrade organic matter. This step is an important in sewage treatment and influences significantly the characteristics of sludge produced (Fernandes, 2001).

The resulting sludge from biological treatment systems of wastewater is composed of live microorganisms. As the efficiency of biological processes is related to the amount of living cells, active in the process, the processing systems keeps the affluent rich in a medium with sludge. A biological process is considered efficient and economical when can be operated with low hydraulic retention times and long retention times of solids to allow the growth of microorganisms. The sludge is thus an initiating factor for the processes of biological treatment of sewage and its excess is regarded as a waste. The efficiency of stabilization and production of this waste depend of the technology of sewage treatment systems and its operationalization (Fernandes 2001).

Stabilisation processes can be divided on biologic and chemist process. Biological methods involve digestion anaerobic, aerobic digestion, autothermal aerobic digestion and composting. The alkaline is the most used chemical process and usually the lime is mixed with the material, improving pH that inactivates some pathogenic microorganisms. Other chemical agents can be chlorine ozone, hydrogen peroxide and potassium permanganate but to a small scale treatment.

4. Composting and parasitic contamination reduction

According to Metcalf & Eddy (1991), composting is an economically and environmentally correct alternative to the stabilization of wastes from agriculture and industries production and urban sewage treatment systems, with possible agronomic use of such waste. This technology involves the organic waste processing into humified composts through two distinct phases. The first corresponds to biochemical reactions of oxidation and the second to the process of humification or maturation phase. Being developed by a microbial ecosystem, the composting is directly influenced by factors that act on microbial activity. Among these, the most important are aeration, temperature, humidity content and nutrient concentration (Veras & Povinelli, 2004).

Large number of treatment plants in Brazil employs the natural composting process, in which organic material, after separation of insensitive material for composting, is arranged in stacks in the courts and are periodically raked to improve aeration. However, there are some cases using expedited procedures with injection of air into the piles (Barrier et al., 2006).

5. Parasitological contamination in compounds with sewage sludge before and after thermal treatment

The temperature is an important factor to contamination reduction in the composting. Study conducted in Brazil had as objective to evaluate the contamination by eggs, cysts and oocysts of protozoa in organic compounds, using sewage sludge and agricultural residues, before and after heat treatment (Duarte et al., 2008). In the experiment conducted in Montes Claros, Minas Gerais, Brazil, 25 different organic compounds were prepared, with approximate volume of 0.5 cubic meters. The materials were kept during the first fifteen days, and daily irrigated and raked (Duarte et al., 2008).

The compounds were prepared with carbon /nitrogen relation of 25:1, 30:1, 35:1, 40:1 and 45:1. In the composition were used cotton waste (5.8 to 10%), seeds of maize and beans (11.8 to 17.2%), and grass seeds (4.4 to 4.7 %). Were also used coconut fiber (50-20%), rice husk (7.4 to 17%), chopped elephant grass (*Pennisetum purpureum*) (1.93 to 17.6%), shell of *Cariocar brasiliense* (pequi) (0.6 to 2.27%), fresh cattle manure (2.3 to 4.8%) and sewage sludge (28.8 to 38.1%). Faeces were from 40 Holstein cows with an average of 5.5 years. Each compound received 220g of rock phosphate, 21kg of gypsum and 21kg of limestone (Duarte et al., 2008). First two samples of 500g sewage sludge were collected from the Juramento Town, in North of Minas Gerais, Brazil. The sludge was collected in the dry bed of the sewage treatment station of this local that uses flow anaerobic reactor ascending. The physical and chemical analysis of sewage sludge were performed according to techniques recommended by Tedesco et al. (1995) and showed pH-H₂O 4.8 and levels of nitrogen, carbon and humidity of 2.4, 9.4 and 6.0%, respectively (Duarte et al., 2008).

Subsequently, were collected 500g samples from compounds with fifteenth day of composting, avoiding contamination from one compound to another and put them in plastic bags, clean and free from contamination by parasites. After this period, the compounds were transferred to a greenhouse with a controlled temperature of 60°C during 12 hours and subsequently to keep 55°C until the twenty-second day after treatment. This procedure was employed with the purpose of promoting the reduction of pathogens parasites of the compounds. The effectiveness of heat treatment and their uniformity was checked in all the

materials produced and three different samples from several parts of these compounds corresponding to the parts A, B and C, were collected after twenty-two days of thermal treatment. Was obtained 500g for each sample (A, B and C), using the same hygienic measures to prevent the contamination (Duarte et al., 2008).

5.1 Processing of samples and parasitological exams

Apart from two samples of sewage sludge was collected in all 100 samples of the produced compounds, with 25 before heat treatment and 75 after treatment (samples A, B and C). The samples were mixed one to one and 10 grams were weighed and stored with 30 ml of 10% formaldehyde solution for preservation of eggs, cysts and oocysts present (Duarte et al., 2008). In the quantification of helminth eggs, protozoan cysts and oocysts, was used the technique of sedimentation of solids during 12 hours after the addition of filtered water to complete one litter. After this procedure, the method Bailenger modified by Ayres & Mara (1996), recommended by WHO was used. Two MacMaster chambers were prepared and for each sample were counted only viable eggs, obtaining the arithmetic mean of the two counts (Ayres & Mara, 1996).

Aiming to check the viability of eggs observed on microscopy was performed quantitative faecal culture technique described by Ueno, (1998). Two grams of the samples before heat treatment and after heat treatment were weighed (corresponding to B samples). The material was mixed with two grams of autoclaved dry sawdust and 10 ml of 1% sulfuric acid solution to fungi growth control. After 28 days of incubation at room temperature, was used the Baermann technique for collection and Keith (1953) key for the identification of larvae from samples obtained before and after heat treatment.

After counting of eggs and larvae of nematodes and protozoa cysts and oocysts from the 25 compounds analyzed, the data were transformed into $\log(X + 1)$ and submitted to the Student's t-test at 5% probability for comparison of the contamination before and after heat treatment.

5.2 Parasitological contamination of sewage sludge

In two s sewage sludge sample from Juramento city, North of Minas Gerais State of Brazil, the results revealed 1.2×10^4 eggs, 10^4 cysts and oocysts/kg for first sample and 2.7×10^4 eggs and 2.7×10^4 cysts and oocysts/ kg of the second sample, respectively. No infective larvae were recovered after quantitative faecal culture, indicating that the process of anaerobic digestion of sewage may have compromised the development and hatching of nematode larvae (Duarte et al., 2008).

In regions with hot weather, the utilization of up flow anaerobic sludge blanket (UASB) is a positive option for the treatment of domestic sewage. However, workers handling in these processes and the produced wastes are potential risk of being infected by parasites (Carvalho et al., 2003; Souza et al., 2006). The effects of aerobic and anaerobic digestion during 15 days on nematode eggs were evaluated by Black et al. (1982). The results indicated that 23% of *Ascaris* spp. eggs were destroyed in the anaerobic treatment and 38% with the aerobic. However, the anaerobic process had no effect on *Trichuris* spp. eggs and none of these treatments was effective for the *Toxocara* spp. eggs.

In a research in the region of Curitiba city, Parana State of Brazil, the contamination of sewage sludge obtained in anaerobic fluidized sludge was evaluated. Were observed significant reductions in the number of viable eggs, present in the material from different

treatment stations, ranging from 59.7 to 93%. The effectiveness of egg reduction was influenced by time and temperature processes and for seasonal effects (Paulino et al., 2001). In another study on the Paraíba State, Northeast of Brazil, was evaluated the effectiveness of the post-treatment. The three investigated systems were wetlands, rock beds and polishing ponds. The results indicated that the raw sewage and UASB effluents from the reactor had averages 353.7 and 50 eggs per litter, respectively. *Ascaris lumbricoides* prevailed on the other species in the raw sewage, with relative frequency of 56.5%. The three systems of post-treatment effluent produced wastes free of helminth eggs and this could be explained by sedimentation produced by a hydraulic surface of 0.20 m day⁻¹ in ponds operated (Souza et al., 2005).

5.3 Parasitic contamination in the compounds before and after thermal treatment

In the table 2 are described the quantification of larvae recovered, counting helminth eggs and protozoan cysts and oocysts in organic compounds before and after heat treatment. These results demonstrate a high parasitic contamination of all the compounds analyzed and statistical analysis indicated no reduction of this contamination after heat treatment (Duarte et al., 2008). All samples were with levels above of one egg per 4 g of compost, which is not recommended for class A biosolids (Fernandes, 2000).

After the larval culture of the compound samples, before of the heat treatment, was observed that 52.4% of the larvae were of the genus *Cooperia*, 36.7% of the genus *Trichostrongylus*, 7.1% *Bunostomum* spp. and 3.8% to *Haemonchus* spp. After heat treatment of the compounds, 33.3% of the larvae were of the genus *Cooperia*, 30.7% of the *Trichostrongylus* spp., 2.7% to infective larvae of *Strongyloides* spp., which are commonly found as parasites of ruminants, and 33.3% of free living forms of the genus *Strongyloides* (Duarte et al., 2008).

The two samples of sewage sludge and the 100 samples of organic compounds showed high counts of viable helminth eggs under light microscopy. However, larvae of the cultures were recovered only in samples from the compounds and the genus identification suggested that the contamination came from the cattle manure or agricultural wastes contaminated with nematode eggs from ruminants (Duarte et al., 2008).

Compounds	eggs x 10 ⁴ /kg		larvae x 10 ⁴ /kg		cysts e oocysts x 10 ⁴ /kg	
	before	after	before	after	before	after
average	33.9	22.3	25.3	41.9	52.8	66.6
standard deviation	31.3	21.2	23.6	71.5	54.7	82.3

(Duarte et al., 2008).

Table 2. Parasitic contamination in 25 different organic compounds produced with sewage sludge and agricultural residues before and after heat treatment at 60 °C for 12 hours in North of Minas Gerais State of Brazil

Table 3 describes the average count of eggs, cysts and oocysts of protozoa from samples of three separate parcels (A, B and C) of each one of the 25 compounds, after the heat treatment at 60°C for 12 hours. There were no significant differences between samples of the same compound ($P < 0.05$). The results revealed high counts of these parasitic structures in both the samples of the compounds after heat treatment, indicating that this process was not effective in the reducing of parasitic contamination in any of the sampled points (Duarte et al., 2008).

The data indicate that, even after composting and thermal treatment, the parasite eggs can remain viable and produce infective larvae. The compounds produced in this study could be classified as class B, for use on crops with little risk of contamination in relation to pathogenic organisms, such as cotton, orchards and plantations. These compounds also should not be applied to pasture for ruminants, since could be the source of contamination for gastrointestinal nematodes (Duarte et al., 2008).

The use of compounds with sewage waste for the fertilization of pastures should also not be displayed, because it could constitute a risk for contamination of the cattle with *Taenia saginata* eggs, contributing to the permanence of both cycles of the bovine cysticercosis and human taeniasis (Duarte et al., 2008).

compound Parcels	eggs x 10 ⁴ /kg			cyts and oocysts x 10 ⁴ /kg		
	A	B	C	A	B	C
average	15.2	22.3	11.0	38.2	66.6	35.8
standard deviation	16	21.2	11.5	51.4	82.3	31.7

Duarte et al. (2008)

Table 3. Contamination with helminth eggs, cysts and oocysts of protozoan in three different samples of organic compounds produced from sewage sludge and agricultural residues after heat treatment at 60 ° C for 12 hours in the North of Minas Gerais, Brazil

6. Different waste treatments and parasitological contamination

Gaspard et al. (1995) also showed that nematode eggs are strongly resistant to most of the classical waste treatments. The work was carried out on sludges from various origins to properly evaluate the impact of the different treatments on nematode eggs. An extraction followed by a concentration procedure allowed isolation of eggs, the viability study being then performed on a culture. For the 19 samples with live eggs, all types of treatment were represented: fresh sludge, prolonged aeration, anaerobic digestion, lagooning, composting and liming. No egg development inhibition phenomenon was observed in fresh sludge. The test demonstrated there were 93% viable eggs. Biological treatments do not produce a total inactivation of nematode eggs. The samples submitted to such various treatments as prolonged aeration, anaerobic digestion, lagooning or composting still showed higher percentages of viable eggs. Prolonged aeration seemed to be totally ineffective with 93%

viable eggs recovered, and a lagoon containing 6-year-old sediments still showed 26% viable eggs. Regarding anaerobic digestion, 66% of viable eggs were recovered in the one sample. For the compost, the analysis on a small number of 8 eggs showed a viability of 25% and the chemical treatment with lime after 20 days of storage gave 66% of viable eggs.

Jhonson et al. (1998) evaluated an in-vitro test for the viability of *Ascaris suum* eggs exposed to various sewage treatment processes. After one week in a mesophilic anaerobic digester, 95% of *A. suum* eggs produced two-cell larvae in vitro, with 86% progressing to motile larvae. After five weeks in the digester, 51% progressed to motile larvae. Between 42% and 49% of eggs stored in a sludge lagoon for 29 weeks were viable and able to develop motile larvae. In the case of eggs that were embryonated before treatment, > 98% survived up to five weeks in the digester and were able to develop motile larvae. More than 90% of embryonated eggs survived for 29 weeks in the sludge lagoon and were able to develop motile larvae.

Solid waste landfill leachate and sewage sludge samples were quantitatively tested for viable *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, *Encephalitozoon hellem*, and *Encephalitozoon cuniculi* spores by the multiplexed fluorescence in situ hybridization (FISH) assay. Depending on the variations utilized in the ultrasound disintegration, sonication reduced the load of human-virulent microsporidian (obligate intracellular parasites) spores to no detectable levels in 19 out of 27 samples (70.4%). Quicklime stabilization was 100% effective, whereas microwave energy disintegration was 100% ineffective against the spores of *E. bieneusi* and *E. intestinalis*. Top-soil stabilization treatment gradually reduced the load of both pathogens, consistent with the serial dilution of sewage sludge with the soil substrate. This study demonstrated that sewage sludge and landfill leachate contained high numbers of viable human-virulent microsporidian spores and that sonication and quicklime stabilization were the most effective treatments for the sanitization of sewage sludge and solid waste landfill leachates (Graczyk et al., 2007).

Kouja et al. (2010) assessed the presence and loads of parasites in 20 samples of raw, treated wastewater and sludge collected from six wastewater treatment plants. Samples were tested by microscopy using the modified Bailenger method (MBM), immunomagnetic separation (IMS) followed by immunofluorescent assay microscopy, and PCR and sequence analysis for the protozoa *Cryptosporidium* spp. and *Giardia* spp. The seven samples of raw wastewater had a high diversity of helminth and protozoa contamination. *Giardia* spp., *Entamoeba histolytica/dispar*, *Entamoeba coli*, *Ascaris* spp., *Enterobius vermicularis*, and *Taenia saginata* were detected by MBM, and protozoan loads were greater than helminth loads. *Cryptosporidium* sp. and *Giardia* sp. were also detected by IMS microscopy and PCR. Six of the eight samples of treated wastewater had parasites: helminths (n=1), *Cryptosporidium* sp. (n=1), *Giardia* sp. (n=4), and *Entamoeba* (n=4). Four of five samples of sludge had microscopically detectable parasites, and all had both genus *Cryptosporidium* sp. and *Giardia* sp. and its genotypes and subtypes were of both human and animal origin.

In another study evaluated the process of anaerobic digestion for treatment of cattle manure. After larvae cultures, positive results were obtained for the L3 larvae of *Haemonchus* spp., *Oesophagostomum* spp. and *Cooperia* spp. in the effluent, even after forty days of retention time (Amaral et al., 2004). However, Padilla & Furlong (1996) observed inactivating effect of anaerobiosis, close to 100%, with the retention time above of 56 days and according to Olson & Nansen (1987), mesophilic anaerobic digestion (35° C) and thermophilic (53° C)

accelerated the inactivation of nematodes in relation to survival time of these parasites in conventional storage.

Sewage sludge and slurry are used as fertilizers on pastures grazed by ruminants. The interest of application on pastures of these two biowastes is environmental (optimal recycling of biowastes) and agronomic (fertilisation). The parasitic risk and the fertilisation value of such applications on pastures were evaluated during one grazing season. The sludge group of calves did not acquire live cysticerci and thus the risk was nil under the conditions of the study (delay of 6 weeks between application and grazing). The slurry group of calves did become lightly infected with digestive-tract nematodes, mostly *Ostertagia ostertagi*. Under the conditions of this experiment, a 6-week delay between application and grazing strongly reduced the risk of infection (Moussavou-boussougou et al., 2005a).

Helminth infection acquired by lambs grazing on pastures fertilised either by urban sewage sludge or cattle slurry were studied by Moussavou-boussougou et al. (2005b) in temperate Central Western France. The aim was to assess the risk of larval cestodosis in lambs after sewage application and of digestive tract nematode infection following the slurry application. The lambs did not acquire cysticercosis or any other larval cestodosis in the sewage sludge group and only very limited infections with *Cooperia* spp. and *Nematodirus* spp. were observed in the slurry group. It was concluded that the helminth risk was extremely low and was not a cause of restriction of the use of these biowastes.

7. Conclusion

The results obtained in the North of Minas Gerais, Brazil, showed that even after the composting of agricultural waste with sewage sludge and heat treatment at 60°C for 12 hours, large numbers of helminth eggs can remain viable. The use of the compounds with sewage sludge should be allocated to perennial crops and low risk of contamination for animals and humans is therefore not recommended for grazing ruminants, for horticulture or for the production of edible mushrooms.

The variation in data of other research to reduce parasitic contamination in composting and anaerobic digestion processes indicates the need for further research, standardizing and monitoring the waste to be recycled for agricultural or other purposes, to reduce risks to public health and animal infection. The initial contamination of sewage sludge used as well as time and temperature of the composting should be elucidated and the final compost produced should always be monitored as to risk of parasitic contamination that could be present.

8. References

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